Primary lymphoedema

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Abstract | Lymphoedema is the swelling of one or several parts of the body owing to lymph accumulation in the extracellular space. It is often chronic, worsens if untreated, predisposes to infections and causes an important reduction in quality of life. Primary lymphoedema (PLE) is thought to result from abnormal development and/or functioning of the lymphatic system, can present in isolation or as part of a syndrome, and can be present at birth or develop later in life. Mutations in numerous genes involved in the initial formation of lymphatic vessels (including valves) as well as in the growth and expansion of the lymphatic system and associated pathways have been identified in syndromic and non-syndromic forms of PLE. Thus, the current hypothesis is that most cases of PLE have a genetic origin, although a causative mutation is identified in only about one-third of affected individuals. Diagnosis relies on clinical presentation, imaging of the structure and functionality of the lymphatics, and in genetic analyses. Management aims at reducing or preventing swelling by compression therapy (with manual drainage, exercise and compressive garments) and, in carefully selected cases, by various surgical techniques. Individuals with PLE often have a reduced quality of life owing to the psychosocial and lifelong management burden associated with their chronic condition. Improved understanding of the underlying genetic origins of PLE will translate into more accurate diagnosis and prognosis and personalized treatment.

Oedema is swelling due to the accumulation of fluid in the interstitium and can involve all parts of the body. Oedema is chronic when it lasts >3 months. The pathophysiological basis for oedema lies upon the forces defined by the Starling law[1] and includes filtration pressure and a colloid osmotic pressure difference between the interstitial fluid and capillary fluids. Oedema develops under abnormal Starling forces, increased endothelial permeability (for example, due to inflammation) or impaired lymphatic drainage[2]. Recurrent comorbidities include venous insufficiency, ulcers, infections (such as cellulitis, a bacterial infection of the skin) and diabetes mellitus[3,4]. Risk factors include age, obesity and heart failure[4]. The careful diagnosis and understanding of the cause of chronic oedema are important for the implementation of a dedicated management protocol and treatment. Today, the term chronic oedema, which was first used for epidemiological purposes[5], is used as an umbrella term for the broader understanding of the term lymphoedema and to cover complex cases of swelling[6–8].

Historically, the term 'primary lymphoedema' (PLE) was recognized in cases of anatomical or functional developmental disorders of the lymphatic system, whereas 'secondary lymphoedema' occurs after the destruction of initially normal lymphatics, for example, by infections (such as filariasis) or invasive surgery. Secondary lymphoedema is the most frequent subtype of lymphoedema, with around 20% of women undergoing breast cancer therapy that includes removal of lymph nodes developing it[4,9]. A clinical approach directed towards (chronic) oedema should consider all (patho) physiological, environmental and personal factors influencing both lymphatic drainage and microvascular filtration[10]. A dedicated article on cancer-associated secondary lymphoedema was recently published in *Nature Reviews Disease Primers*[11].

The focus of this Primer is on PLE, an umbrella term that covers all developmental lymphatic anomalies leading to a failure of the lymphatic system and swelling of any part of the body (FIG. 1). PLE can be congenital or develop later in life (at puberty or even beyond 50 years of age). Diagnosis of PLE can be difficult and many individuals remain undiagnosed. There are no good incidence or prevalence estimates and even less so regarding geographical regions and ethnicities. LIMPRINT, an international consortium, is trying to establish such data[12]. In the USA, 165,000 lymphoedema-related admissions were recorded between 2012 and 2017 (REF[13]). The classification of PLE has long been based on the age of onset (congenital, early onset or late onset); however, with the discovery of underlying genetic causes, a gene and symptom-based classification has been proposed

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by the International Society of the Study of Vascular Anomalies (ISSVA). We follow this classification, which is based on clinical and genetic findings. The newly suggested dyadic nomenclature associating gene name with a phenotypic descriptor could provide a more precise nomenclature (for example, VEGFR3 (encoded by *FLT4*)-related lymphoedema)[14]. An algorithm that helps with diagnostic workup has also emerged (see Diagnosis, screening and prevention)[15]. PLE often occurs isolated but it can also be associated with a variety of additional clinical features. As of December 2020, an OMIM query with the term "lymphedema" retrieved 94 entries, underscoring the strong genetic influence. Current treatments of PLE are often limited to alleviating symptoms or surgery. Thus, there is an important need for improved patient care. This need calls for a better understanding of the underlying causes of PLE to enable the development of novel treatments.

Epidemiology

Lymphoedema has been known since the middle of the nineteenth century. Hereditary forms were first reported by Nonne[16] and Milroy[17]; these forms were congenital PLE. In 1898, Meige reported an inherited form of puberty-onset PLE. PLE associated with yellowing of the nails was reported in 1964 (REF[18]). The cause of Meige disease and yellow nail syndrome still remain unknown[19]. The first inherited mutations causing PLE were discovered for the so-called Nonne–Milroy disease in 2000 in the *FLT4* gene, encoding vascular endothelial growth factor receptor 3 (VEGFR3), followed by the discovery of many other mutations[20,21]. Altogether, 31 genes or loci have been reported to cause postnatal PLE with or without preceding non-immune hydrops fetalis (NIHF; severe prenatal oedema) (TABLE 1) and 18 await further confirmation (TABLE 2). These genes or loci explain about 27% of PLE cases in one well-studied cohort[22,23]. Whether there are differences between ethnicities is unknown. In some phenotypes, peripheral PLE is associated with central lymphatic defects otherwise called complicated lymphatic anomalies (CLAs)[24]. In addition to genes mutated in PLE, an increasing number of genes is associated with NIHF[25]. NIHF is often recessive and can be lethal. It can be observed in association with lysosomal storage diseases, skeletal dysplasias, cardiac anomalies and disorders of glycosylation. Although not yet reported, variants in genes associated with NIHF may also be associated with PLE, increasing further locus heterogeneity behind PLE.

The prevalence of PLE has been estimated at 1.5 per 100,000 individuals in older studies[26]. However, this figure was based on a retrospective study using the database of recorded diagnoses extrapolated to the population and it most likely underestimates the prevalence of PLE. Recent large-scale estimates of the prevalence of chronic oedema (including PLE and secondary lymphoedema) in the South West London community vary from a general prevalence of 1.33 per 1,000 people in the population of the catchment area, increasing to 5.4 per 1,000 individuals aged >65 years and 10.3 per 1,000 individuals aged >85 years[5]. One study evaluated a database of 9,477 patients with lymphoedema between 1999 and 2010, of whom 138 had an age of onset of <21 years (2.6% of the lymphoedema population)[27]. Others described PLE as a predominantly paediatric disorder affecting 1.2 per 100,000 people aged <20 years[26]. Clearly, better definitions and more accurate numbers are still missing.

One reason for the lack of precise epidemiology is also the important heterogeneity in clinical presentation of lymphoedema (TABLES 1,2). Moreover, each phenotype is a rare disease, meaning that the prevalence is below the threshold of 5 in 10,000. In addition, penetrance in familial cases can be low, as is the case, for example, for mutations in *VEGFC* (encoding vascular endothelial growth factor C (VEGFC)) in Milroy-like disease, in which up to 50% of individuals carrying a mutation do not develop clinically detectable lymphoedema[28].

Some studies suggest that PLE is more frequent in women, for example, in families with a *CELSR1* mutation[29]. This observation suggests that hormonal differences may have a role. However, there might be other biases, such as the often-referred fact that women tend to be more prone to consulting clinicians than men (owing to stronger societal pressure on physical appearance) and/or that PLE is more severe in females than in males. Imaging of the lymphatic system in relatives in the same families who carry PLE mutations sometimes unravels abnormal lymphatic vasculature also in mutation-carrier individuals who have not (yet) developed lymphoedema[30].

Our current knowledge on the genetic variability among PLE remains limited. For about 70% of patients, an underlying genetic defect has not yet been discovered but is probably present in many[22,23] and most of the genes with Mendelian mutations concern a limited number of patients and families (only one or two patients or families have been reported for some genes, such as *PTPN14* (REF[31]), *RELN*[32] or *GJA1* (REFS[33,34])). Several of the genes have also been identified only recently, limiting epidemiological data. Moreover, in some conditions, such as microcephaly with or without chorioretinopathy, lymphoedema or mental retardation syndrome due to mutations in *KIF11*, lymphoedema is not always present[35]. This finding renders it difficult to have a representative and comprehensive overview of the current state of the epidemiology of PLE as a whole and even more so for each of the subtypes.

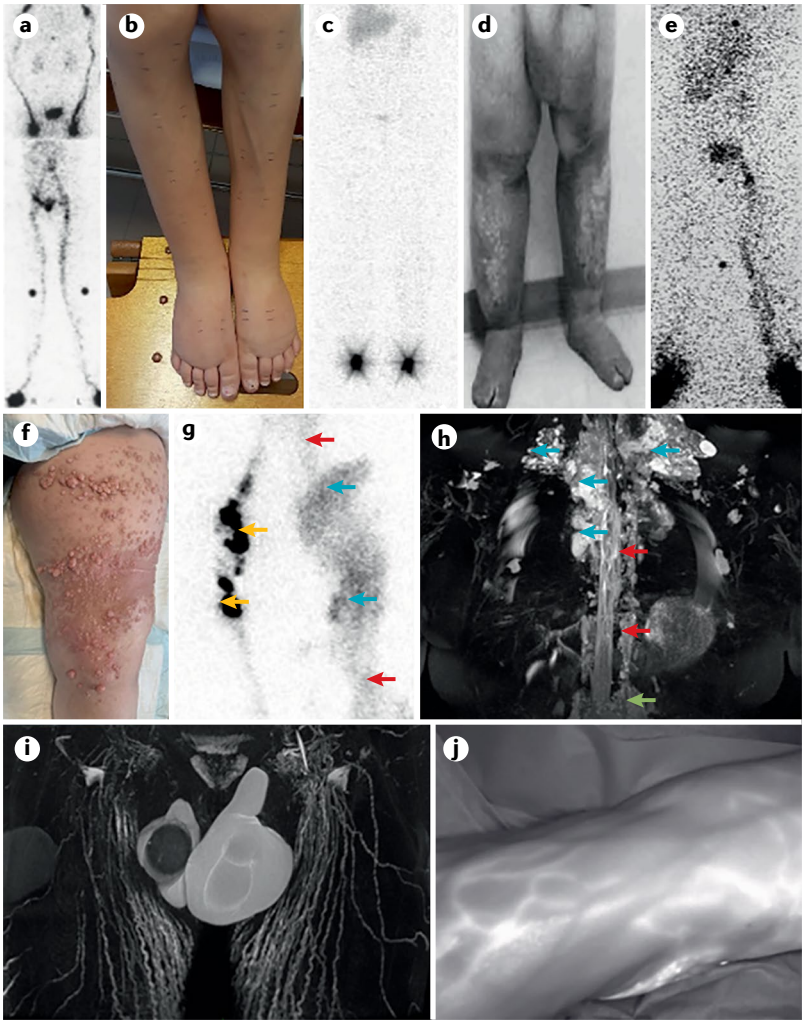

Fig. 1 | **Examples of primary lymphoedema.** Technetium (^{99m}Tc) albumin-aggregated lymphoscintigraphy (LSG) of hands (top) and feet (bottom) of a healthy person as a control (part **a**). Bilateral primary lymphoedema (PLE) in an individual carrying a *VEGFR3* mutation, with visible large saphenous vein on top of the fascia (part **b**) and LSG showing no uptake (part **c**). Familial hyperplastic refluxing PLE on lower extremities in an individual carrying a *FOXC2* mutation (lymphoedema–distichiasis) (part **d**), with LSG showing associated genital lymphoedema and irregular poorly transporting lymphatic collector channels on the left, with extensive dermal and genital tracer reflux after bilateral foot injections (part **e**). Congenital PLE with lymphatic hyperplasia involving the left lower extremity (part **f**), with LSG showing lymph stasis visible on left abnormal lymphatic nodes (blue arrows) and enlarged trunks (red arrows) as compared with normal right nodes and trunks (yellow arrows) (part **g**). Lympho-MRI showing central conducting lymphatic anomalies with enlargement of the Cysterna chyli (green arrow) and thoracic duct (red arrows), and large lymphatic anomalies on both sides of the upper mediastinum and upper thorax (blue arrows) (part **h**) similar to those in part **g**. Examples of superficial lymphatic hyperplasia visualized by lympho-MRI (part **i**) and visualization of superficial lymphatic network on the arm by injection of indocyanine green (part **j**).

The European Commission stated that "rare diseases are life-threatening or chronically debilitating diseases that are of such low prevalence that special combined efforts are needed to address them"[36]. The International Lymphoedema Framework considers lymphoedema as a neglected health problem and has set up the LIMPRINT study[12]. Although predominantly studied in Caucasians, PLE has been reported in Asians[37] and Africans[38,39]. Guidelines for PLE care have also been established for South African clinicians[40]. In Europe, expert referral centres have been nominated by health ministries in different EU countries and networked under 24 European Reference Networks to gather the best expertise and provide accessible cross-border health care. The European Reference Network of Rare Vascular Diseases (VASCERN) covers the working groups on primary and paediatric lymphoedema (PPL-WG) and on vascular anomalies (VASCA-WG). These working groups define recommendations for diagnostics, prevention, and treatment[41] and can also endorse published national guidelines[42]. An important patient/professional organization supporting the field is the Lymphatic Education & Research Network, in addition to the longstanding involvement and programmes of the International Society of Lymphology (ISL), founded in 1966, and the National Lymphedema Network, founded in 1987.

Mechanisms/pathophysiology

Lymphatic vessel malfunction

Normal lymphatic physiology. The lymphatic system is a unidirectional vascular system that transports surplus tissue fluid back to the blood circulation. The system is composed of vascular conduits and lymphoid organs, including the lymph nodes and cellular elements such as lymphocytes and dendritic cells circulating in liquid lymph. Lymph also contains 'absorbents' such as water and chylomicra, which are lipoproteins formed in the small intestine that transport dietary fats.

For lymphatic vessels to function effectively, lymph — formed from blood capillary filtrate, cell products and trafficking cells — must be absorbed from the interstitium, carried through non-leaky, valveless initial lymphatics and pre-collectors, and propelled through patent, intrinsically contractile, valved peripheral collecting channels and interposed lymph nodes to finally reach the cisterna chyli (a dilated collecting structure) that drains visceral lymph, including milky chylous intestinal lymph in the abdomen (FIG. 2). From there, lymph from the lower part of the body finally arrives at the main thoracic duct. In addition, although with considerable variability in the topographical anatomy of these central lymph-collecting structures, a right lymphatic duct also drains the lungs, heart and upper right quadrant of the body. These collectors are joined by the bilateral cervical lymphatics draining lymph from the head and neck. Cervical lymphatics also drain lymph from the specialized meningeal lymphatic system (which collects interstitial, cerebrospinal and perivascular fluid from the brain) and the glymphatic drainage in the brain[43,44]. This central lymph then passes through bilateral valved entries (lymphovenous valves) into the central venous system to complete the 'blood–lymph loop' of the extracellular fluid circulation[45]. Of note, the lymphatic channel pathways are much more variable inter-individually than those of arteries or veins[46–48].

The process of lymph formation (termed the lymphatic load) is governed by two gradients: the gradient of hydrostatic pressure from fluid within the blood capillaries, which forces fluid outward to the lower pressure of the interstitial fluid, and the inward gradient of oncotic pressure due to plasma proteins and other large

Table 1 | **Confirmed lymphoedema genes and loci**

Gene[a]	Locus	Disease or syndrome	Major associated signs	OMIM number	Inheritance	Protein function[b]	Refs
(Prader–Willi)	15q11.2	Prader–Willi	Obesity, developmental delay, short stature	176270	AD	NA	233
(Aagenaes)	15q26.1	Cholestasis–lymphoedema	Cholestasis	214900	AR	NA	121
(*TBX1*?)	22q11.2 del	22q11 deletion	Dysmorphism, cardiovascular anomalies	611867	De novo	NA	120
(Phelan–McDermid)	22q13 del	Phelan–McDermid	Developmental delay, hypotonia	606232	AD	NA	168
(Turner)	Xp11.4/ Yp11.2 del	Turner	Cardiac anomalies, webbed neck, dysmorphism, slowed growth	NA	De novo	NA	114,133
ABCC9	12p12.1	Cantu	Hypertrichosis, osteochondrodysplasia, cardiomegaly	NA	AR	Anion transporter	234
ADAMTS3	4q13.3	Hennekam lymphangiectasia–lymphoedema syndrome 3	Dysmorphism, protein-losing enteropathy	618154	AR	Extracellular enzyme	93,235
ANGPT2	8q23.1	Lymphoedema	NA	NA	AD	Ligand	23
CCBE1	18q21.32	Hennekam lymphangiectasia–lymphoedema syndrome 1	Dysmorphism, protein-losing enteropathy	235510	AR	Adaptor protein	236
CELSR1	22q13.31	Lymphoedema	NA	NA	AD	Transmembrane	104
EPHB4	7q22.1	Hydrops fetalis, central conducting lymphatic anomaly (HFASD)	Hydrops fetalis	617300	AD	Transmembrane receptor	109,237
FAT4	4q28.1	Hennekam lymphangiectasia–lymphoedema syndrome 2	Dysmorphism, protein-losing enteropathy	616006	AR	Transmembrane protein	238
FLT4 (*VEGFR3*)	5q35.3	Nonne–Milroy lymphoedema	Hydrops fetalis	153100	AD, AR, de novo	Transmembrane receptor	20,21
FOXC2	16q24.1	Lymphoedema–distichiasis	Distichiasis, ptosis, varicose veins	602402	AD, de novo	Transcription factor	73
GATA2	3q21.3	Emberger	Myelodysplasia	614038	AD	Transcription factor	171
GJC2 (*Cx47*)	1q42.13	Lymphoedema	NA	613480	AD	Connexin	108
IKBKG (*NEMO*)	Xq28	Osteopetrosis with lymphoedema	Dental anomalies, ectodermal dysplasia, immunodeficiency	300291	X-linked	Intracellular signalling molecule	239,240
KIF11	10q23.33	Microcephaly with or without chorioretinopathy, lymphoedema or mental retardation	Microcephaly with or without chorioretinopathy, mental retardation	152950	AD, de novo	Intracellular signalling molecule	241
KRAS	12p12.1	Noonan syndrome 3, Gorham–Stout disease	Short stature, dysmorphism, cardiac anomaly, developmental delay	609942	AD, de novo	Intracellular signalling molecule	242,243
NSD1	5q35.3	Sotos syndrome 1	Macrocephaly, rapid growth, cardiac anomaly	117550	De novo	Histone methyltransferase	244,245
PIEZO1	16q24.3	Hydrops fetalis, generalized lymphatic dysplasia	Hydrops fetalis, short stature, facial dysmorphism	616843	AR	Ion channel	246,247
PTPN11 (*SHP2*)	12q24.13	Noonan syndrome 1, hydrops	Short stature, dysmorphism, cardiac anomaly, developmental delay	163950	AD	Intracellular signalling molecule	248
RAF1	3qp25.2	Noonan syndrome 5	Short stature, dysmorphism, cardiac anomaly, developmental delay	611553	AD	Intracellular signalling molecule	249,250
RASA1	5q14.3	Parkes–Weber (CM-AVM1), chylothorax	Capillary and arteriovenous malformations	139150	AD	Intracellular signalling molecule	251,252,253
RIT1	1q22	Noonan syndrome 8	Short stature, dysmorphism, cardiac anomaly, developmental delay	615355	AD	Intracellular signalling molecule	254–257
SOS1	2p22.1	Noonan syndrome 4	Short stature, dysmorphism, cardiac anomaly, developmental delay	610733	AD	Intracellular signalling molecule	258,259

Table 1 (cont.) | **Confirmed lymphoedema genes and loci**

Gene[a]	Locus	Disease or syndrome	Major associated signs	OMIM number	Inheritance	Protein function[b]	Refs
SOS2	14q31.1	Noonan syndrome 9	Short stature, dysmorphism, cardiac anomaly, developmental delay	616559	AD	Intracellular signalling molecule	260–262
SOX18	20q13.33	Hypotrichosis–lymphoedema–telangiectasia–(renal defect) syndrome	Hypotrichosis, telangiectasia, ileal atresia, aortic dilation	607823, 137940	AD, AR, de novo	Transcription factor	228,263
THSD1	13q14.3	Hydrops, severe oedema	Hydrops fetalis, cardiac anomaly	NA	AR	Transmembrane protein	264,265
TSC2	16p13.3	Tuberous sclerosis 2	Hamartomas, developmental delay	191092	AD	Intracellular signalling molecule	266,267
VEGFC	4q34.3	Nonne–Milroy-like lymphoedema	NA	615907	AD	Ligand	28,134, 268–270

OMIM numbers are only provided if the description mentions a lymphatic defect. When a maximum of five publications exists for primary lymphoedema-causing mutations in one gene, we listed them all; otherwise, if more than five, we listed only the original ones. AD, autosomal dominant; AR, autosomal recessive; CM-AVM1, capillary malformation-arteriovenous malformation 1; NA, not applicable. [a]Mutated genes and loci associated with postnatal primary lymphoedema with or without non-immune hydrops fetalis with more than three index patients reported or, if only two index patients, supported by functional validation or linkage; in alphabetical order. [b]See signalling in FIG. 3.

molecules that cannot pass freely through the capillary barrier, which drives fluids towards the blood vessels and is therefore opposed by the hydrostatic pressure gradient. These so-called Starling forces and the lymphatic load are further modified by a filtration coefficient reflecting capillary surface area and permeability. Normally, in an oedema-free state, the volume of the lymphatic load is matched by the lymphatic capacity (the rate of lymph adsorption) to return lymph to the bloodstream (normal thoracic duct lymph flow is approximately 1 ml per minute)[45,49–52]. There are regional variations in the absolute numerical value of the Starling forces and filtration coefficient (for example, the tight relatively impermeable blood–brain barrier in contrast to the low-hydrostatic pressure, highly permeable liver sinusoids) under normal physiological conditions, which may be greatly exaggerated in disease states[53,54].

Pathophysiology of PLE. Congenital lymphatic malformation or malfunction anywhere along these continuous pathways can be fatal during prenatal life or delayed or even silent after birth, until it leads to an imbalance between the processes of lymph formation (lymphatic load) and lymph absorption (lymphatic capacity)[45,49–51] (FIG. 2). At that point, tissue swelling becomes manifest, presenting as PLE involving the limbs, chylous (chylomicra-containing intestinal lymph) or non-chylous lymph accumulations and/or effusions in the body cavities, as lymphostatic encephalopathy from brain oedema[33], or even as external leaks (in which lymph exudates from the skin). Stasis of lymph (a high protein, hyaluronan-rich fluid-altering extracellular matrix (ECM)) reflects diminished lymphatic capacity and sets into motion a localized tissue response. This response is characterized in varying degrees by inflammation, fibrosis, adipose deposition, immune dysregulation, susceptibility to infection, and both lymphangiogenesis and haemangiogenesis as part of a progressive 'overgrowth' phenomenon[45,49–51,55].

At the absorptive level, aberrations that could interfere with the relatively free passage of fluid and macromolecular and cellular lymph components into the lymphatic capillaries include alterations in initial interendothelial open 'buttons' (loosely apposed permeable junctions) and continuing closed impermeable 'zipper' junctions[56] and related gap junction proteins, anchoring filaments[45,49,50], tissue pressure mechanosensors (for example, Piezo-type mechanosensitive ion channel component 1 (PIEZO1))[57], and the newly described hyaluronan bulbs[58] (structures composed of a large matrix of glycosaminoglycan and integrins, which are involved in cellular migration into the lymphatics dependent on lymphatic vessel endothelial hyaluranon receptor 1, Lyve1)[59] (FIG. 2). Disturbances in lymphatic vessel growth (either reduced growth (aplasia or hypoplasia), increased size or number of the vessels (megalymphatics and hyperplasia, respectively), or growth in the wrong place, termed collectively as 'lymphangiodysplasias') could lead to or reflect anatomical or functional lymphatic obstruction (peripheral and/or central). These abnormalities can be imaged dynamically most easily in PLE by whole-body lymphoscintigraphy (particularly when combined with resolution-enhanced and 3D-localized single-photon emission computed tomography (SPECT)) but also by other modalities such as MRI with or without contrast and indocyanine green (ICG) fluorescent lymphangiography[45,47–50,55,60–66]. Lymphangiodysplasias can also be caused or exacerbated by maldeveloped, hypoplastic or fibrotic regional lymph nodes[67]. Defective lymphatic valves[45,48–50,55,60,68] can lead to valve incompetence, lymphangiectatic dilatations and lymph reflux into superficial valveless collaterals, tissues or body cavities or as external leakage from the skin. Heightened permeability can allow leakage from initial or collecting lymphatics and impaired contractility[45,48–50,55] would delay lymph transport. Specific transgenic mouse models closely mimic these contrasting clinical and lymphatic imaging phenotypes (for example, lymphatic aplasia or hypoplasia in the angiopoietin 2 (*Angpt2*) knockout mouse[69] and refluxing lymphatic hyperplasia in the *Foxc2* haploinsufficient mouse[70]).

These pathogenetic mechanisms may stay latent and not manifest as tissue fluid accumulation and, moreover, they might affect other cardiovascular or general systemic

Table 2 | **Suggested lymphoedema genes**

Gene[a]	Locus	Disease or syndrome	Major associated signs	OMIM number	Inheritance	Protein function[b]	Refs
ARAF	Xp11.3	Central conducting lymphatic anomaly	NA	NA	De novo	Intracellular signalling molecule	190
B3GAT3	11q12.3	Linkeropathies	Short stature, skeletal dysplasia, dysmorphism, cardiac anomaly, developmental delay	NA	AR	Extracellular enzyme	271
BRAF	7q34	Noonan syndrome 7 and cardiofaciocutaneous syndrome	Short stature, dysmorphism, cardiac anomaly, developmental delay	613706	AD	Intracellular signalling molecule	272,273
CBL	11q23.3	Noonan syndrome-like disorder with or without leukaemia	Short stature, dysmorphism, cardiac anomaly, developmental delay	613563	AD	Intracellular signalling molecule	274
FBXL7	5p15.1	Hennekam	Dysmorphism, protein-losing enteropathy	NA	AR	Ubiquitin protein ligase complex subunit	275
GJA1 (*CX43*)	6q22.31	Oculodentodigital dysplasia	Microcephaly, hearing loss, dysmorphism	164202	AD	Connexin	33,34
HGF	7q21.11	Lymphoedema	NA	NA	AD	Ligand	96,276
KLHL40	3p22	Hydrops fetalis	Myopathy	615348	AR	E3-ubiquitin ligase	277,278
MET	7q31	Lymphoedema	NA	NA	AD	Transmembrane receptor	96,276
NF1	17q11.2	Neurofibromatosis type 1	Cafe-au-lait spots	162200	AD	Intracellular signalling molecule	279
NRP1	10p11.22	Lymphoedema	NA	NA	AD	Transmembrane co-receptor	280
NRP2	2q33.3	Lymphoedema	NA	NA	AD	Transmembrane co-receptor	280
PTPN14	1q32.3–41	Choanal atresia and lymphoedema	Choanal atresia	613611	AR	Intracellular signalling molecule	31
RELN	7q22.1	Lissencephaly 2	Lissencephaly	257320	AR	Extracellular matrix protein	32
SEMA3A	7q21.1	Lymphoedema	NA	NA	AD?	Extracellular matrix protein	281
SHOC2	10q25.2	Noonan syndrome-like disorder with loose anagen hair 1	Short stature, dysmorphism, cardiac anomaly, developmental delay	607721	AD	Intracellular signalling molecule	282,283
TIE1	1p34.2	Lymphoedema	NA	NA	AD	Transmembrane receptor	284
TSC1	9q34.13	Tuberous sclerosis 1	Hamartomas, developmental delay	191100	AD	Intracellular signalling molecule	285,286

OMIM numbers are only provided if the description mentions a lymphatic defect. When a maximum of five publications exists for primary lymphoedema (PLE)-causing mutations in one gene, we listed them all; otherwise, if more than five, we listed only the original ones. AD, autosomal dominant; AR, autosomal recessive; NA, not applicable. [a]Genes suggested on the basis of only one or two patients: rare cases of known syndrome with PLE or associations on the basis of a limited number of suggestive variants. Some could have PLE by coincidence and/or another cause. These await confirmation by identification of additional cases and/or functional validation; in alphabetical order. [b]See signalling in FIG. 3.

developmental events, resulting in multiorgan syndromes or, in select instances, contributing to lymph overproduction (overload), for example, by venous pressure elevation, further overwhelming the diminished lymphatic capacity. Indeed, specific initiating mechanisms controlling lymphatic growth, specialized lymphatic structures, and cell migration and adhesion (at an anatomical, physiological or molecular level) have been pinpointed in some forms of PLE (see Genetic basis below). These mechanisms have been observed in patients and/or mouse models and further elucidated in vitro. However, exactly how lymph transport is affected and compensated for (at the peripheral, visceral and central lymphatic system level) and what accounts for the variability in manifesting lymphoedema (site, severity, age of onset) remains elusive. Current techniques for non-invasive in vivo multimodal lymphatic imaging and histological study of diseased lymphatics are limited in assessing lymphodynamics. Yet, determining the sequence, interaction and impact of specific functional abnormalities in the integrated lymphatic system of vessels, fluid, nodes and trafficking of immune cells (lymphatic 'systemomics')[71] is particularly crucial to fill in the gaps in our understanding of uncomplicated PLE and of the pleiomorphic manifestations in associated syndromes[55,72] and to translating the findings of experimental models into the management of the human condition.

Genetic basis

Single genes. Our evolving knowledge of genes in which mutations cause lymphoedema has depended on synergistic findings in both human and mice. Zebrafish have more recently been studied and it is clear that the relative importance of the many shared essential gene products can differ between the three species. The first major discoveries were based on classical human genetic approaches using linkage studies in large families to locate and clone genes involved in lymphatic

development and function. Germline mutations in *FLT4* in Nonne–Milroy disease and *FOXC2* in lymphoedema–distichiasis (development of extra eyelashes) syndrome are early examples[20,21,73] (TABLE 1). The finding of lymphatic abnormalities in mice with mutations in *Prox1*, *Angpt1* and *Angpt2* led to the discovery of genes involved in the early stages of lymphatic development. The many genes involved and their mechanisms of action have been recently reviewed[74–76]. Overall, three patterns of inheritance are observed in PLE, including autosomal dominant (including de novo mutations), autosomal recessive and X-linked (TABLES 1,2). PLE mutations affect proteins with various types of cellular function; most mutations are loss-of-function, although some, such as in the genes associated with Noonan syndrome, are gain-of-function.

Initiation of lymphatics. The first theory on lymphangiogenesis proposed that primitive lymph sacs (primordial lymphatic vascular structures) arise from endothelial cells, which are derived from embryonic veins, and assemble to form lymphatic capillaries[77]. An alternative theory suggested that lymph sacs are derived from lymphangioblasts, that is, mesenchymal precursor cells independent of veins, in a process similar to vasculogenesis[78,79]. In fact, lymphangiogenesis seems to be a combination of both. According to the second theory, lymphangioblasts, the first lymphatic endothelial precursors, likely differentiate in part from a subset of endothelial cells located in lateral regions of the anterior cardinal vein and/or at least in part from undifferentiated mesenchymal cells in peripheral tissues[80]. Lymphangioblasts sprout, migrate and proliferate to form lymph sacs. Centrifugal sprouting from these sacs forms distinct lymphatic capillary networks, which later merge to develop the primitive lymphatic capillary plexus[81]. The paraxial mesoderm was also demonstrated as a major source of lymphatic endothelium[82].

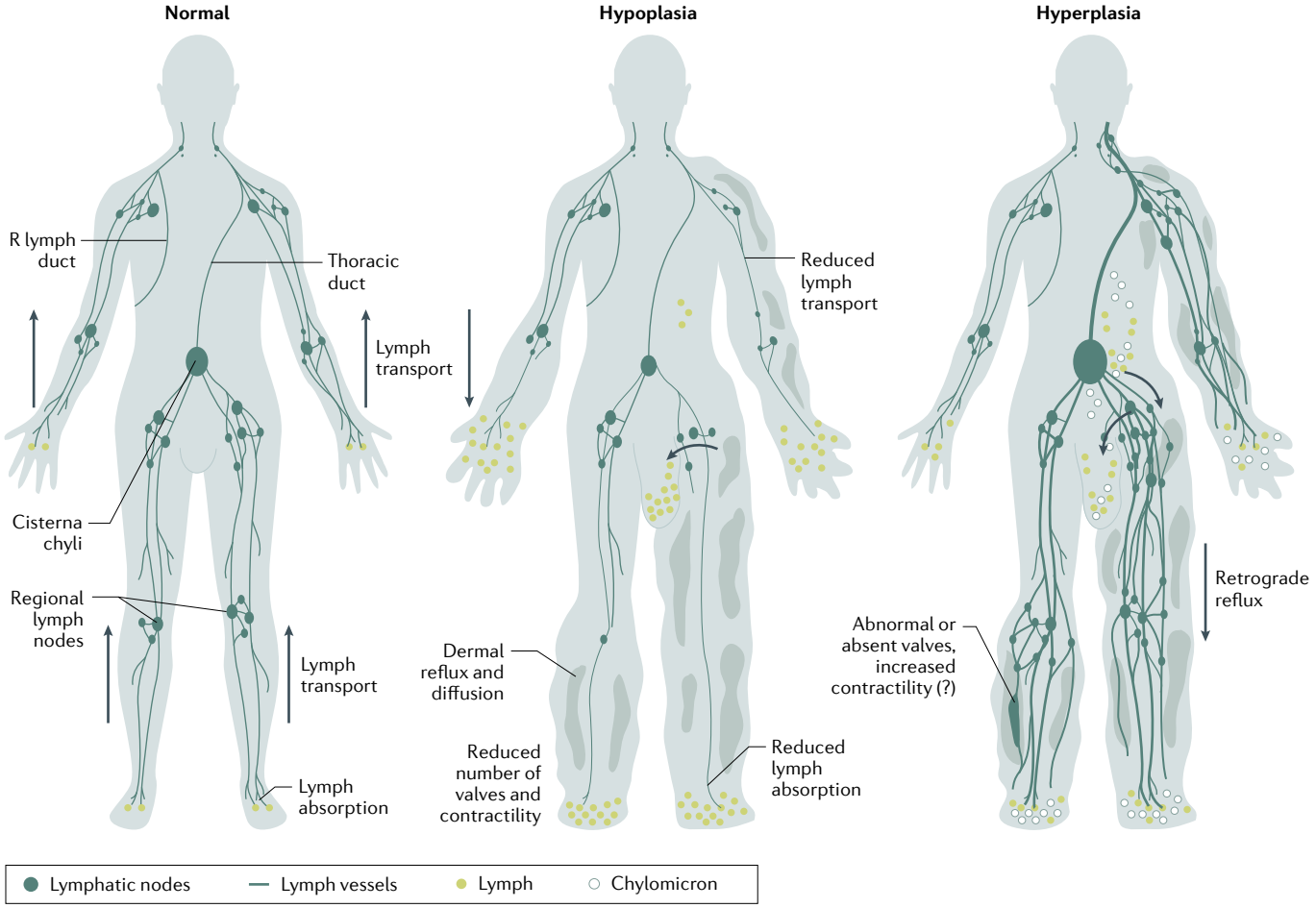

Fig. 2 | **Schematic of the spectrum of pathological findings in PLEs.** Primary lymphoedema (PLE) can be classified into hypoplastic and hyperplastic forms on the basis of various lymphatic imaging modalities and operative findings. On the left side of the hypoplastic and hyperplastic examples are mild segmental forms and, on the right, more generalized (systemic) forms. Individual patients may exhibit a single feature or combinations of these findings. In general, pathogenetic gene variants, exemplified by the two most common hereditary lymphoedemas, fall into two categories. The first involves deficiencies of lymphatic growth factor ligands or receptors (for example, mutations in the gene encoding VEGFR3), which impair the growth of lymphatic channels and associated lymph nodes and manifest as hypoplasia. The second type interferes with lymphatic valve formation or function (for example, mutations in the gene encoding FOXC2) and is associated with hyperplasia of lymphatic channels and nodes and retrograde lymph flow. Over time, as lymphoedema persists, additional non-specific changes occur such as lymphatic and lymph node fibrosis or obliteration, pericyte investment of lymphatic capillaries, and exuberant lymphangiogenesis.

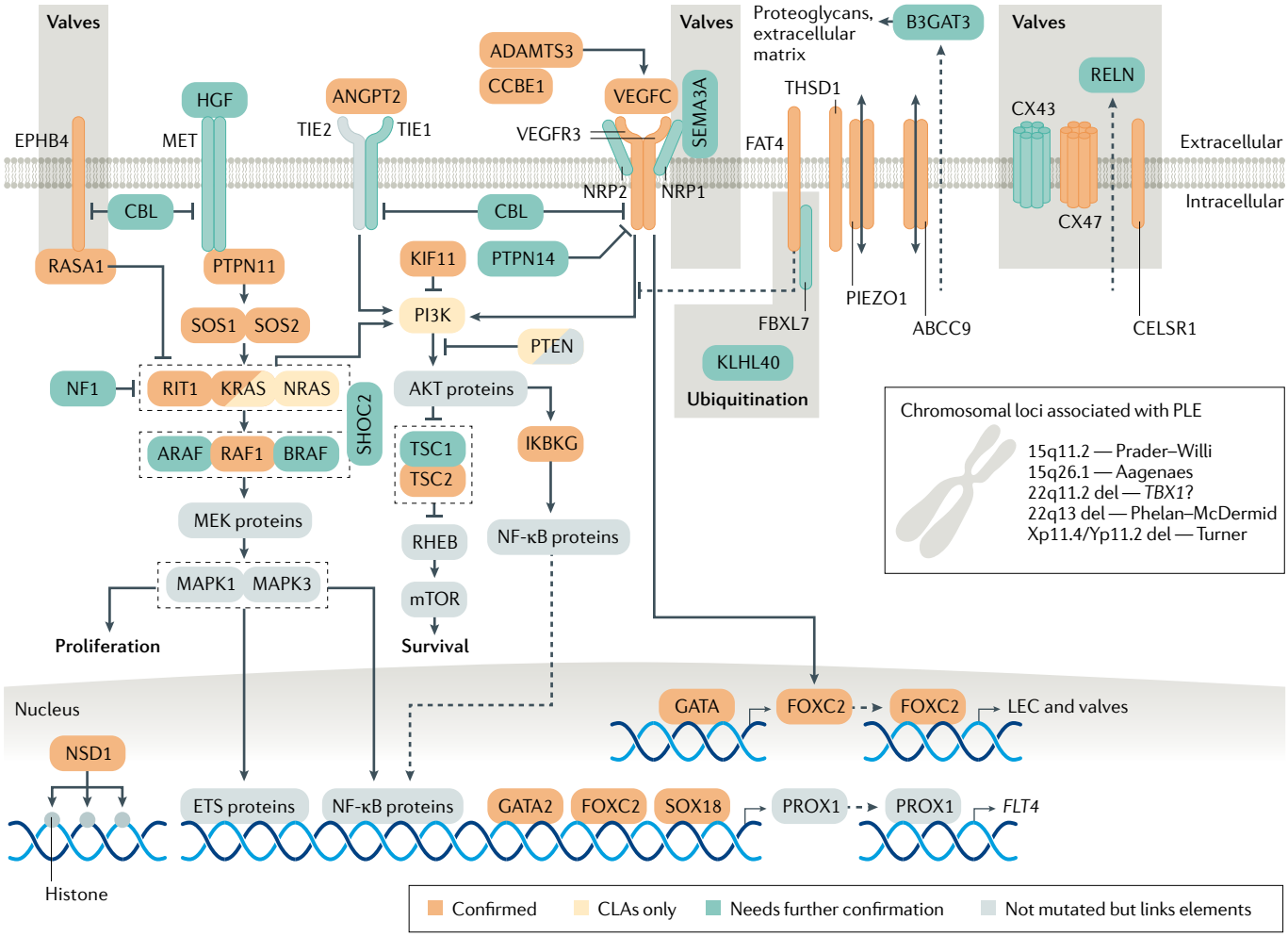

Fig. 3 | **Loci, genes and proteins associated with PLE.** The core of the lymphatic pathway is constituted by the VEGFC–VEGFR3 axis, but additional ligand–receptor signalling pathways emerge, including ANGPT2–TIE1 or ANGPT2–TIE2 and HGF–MET. Phenotypes with a mutation in one of the proteins of the RAS pathway constitute the RASopathies. Genes and proteins in orange have been confirmed to be associated with primary lymphoedema (PLE), whereas those in green need to be confirmed in additional patients or by functional validation of the variants. Genes associated with complicated lymphatic anomalies (CLAs) are in yellow; most variations in these are somatic or mosaic but *KRAS* mutations are also germline in PLE (Noonan) and a germline *PTEN* mutation was reported once in Gorham–Stout disease. Accessory proteins not known to be mutated in PLE but part of the pathways are in grey. Dashed lines indicate protein transfer or secretion or indirect inhibition. LEC, lymphatic endothelial cell.

There are multiple genes involved in the early development of the lymphatics, mainly studied in mice and zebrafish, and some of these genes have been found to be implicated in human lymphatic disease; not surprisingly, some of them are also involved in vasculogenesis. The first human lymphatic-specific markers were VEGFR3 (REF.[83]), LYVE1 (REF.[84]) and podoplanin[85]. The discovery of prospero homeobox protein 1 (PROX1)[86] provided the earliest expressed marker for early lymphatic development. Its absence prevented lymphatic endothelial cell (LEC) development, which instead became blood endothelium and resulted in only a blood vascular phenotype. Its continuous expression is required to maintain the LEC phenotype[87]. The upregulation of *Prox1* (reviewed in REF.[88]) induced many LEC markers such as podoplanin and VEGFR3, many transcription factors (especially FOXC2 but also IKBKG, GATA2, SOX18 and KIF11; mutations in the genes encoding the corresponding human proteins cause lymphoedema-associated syndromes in patients; TABLE 2), receptors, cell cycle regulators, and adhesion factors (their complex interactions will be further described and are illustrated in FIG. 3)[89]. The ECM composition also has an important role and can regulate transcription factor activity[90].

Besides signalling molecules, membranous receptors and transcription factors, other bioactive molecules, such as retinol for lymphatic maturation, are essential[91]. ECM components, such as SVEP1 (also known as polydom), also have a role[92]. Hennekam lymphangiectasia (abnormal dilation of lymphatic vessels)–lymphoedema syndrome is caused by mutations in multiple genes, such as those encoding the ECM-binding protein CCBE1, ADAMTS3 (an enzyme that cooperates with CCBE1 to cleave and activate VEGFC) and FAT4 (a membrane protein likely to be involved in cell polarity)[93,94].

Rare mutations in *VEGFC*, the major stimulator of lymphatic growth and development, also cause lymphoedema[28]. Its receptor, VEGFR3, is a tyrosine kinase receptor initiating PI3K–AKT signalling and the interacting RAS–MAPK cascade. Inactivating mutations in *VEGFR3* are a cause of Nonne–Milroy disease (familial congenital bilateral lower-limb lymphoedema, the most frequently found genetic cause of lymphoedema)[20,21] (FIG. 3; TABLE 2). Mutations in the genes encoding many components of these intracellular signalling pathways are associated with phenotypes exhibiting lymphoedema, including Noonan syndrome, cardiofaciocutaneous syndrome, lymphoedema–choanal atresia syndrome and rare cases of chylothorax (thoracic duct damage with chyle leakage surrounding the lungs) (see Signalling pathways below; FIG. 3; TABLES 1,2). Mutations in some of these genes can serve as modifying genes; for example, mutations in *GJC2* (encoding connexin 47) may interact with non-genetic factors to cause post-breast surgery lymphoedema[95] or be causative in PLE[96].

Lymphatic valve formation. In general, mutations in genes related to defective valve formation tend to produce lymphatic hyperplasia or lymphangiectasia and lymph reflux. By contrast, genes involved in the initiation (above) or maintenance and proliferation (below) of lymphatics produce PLE with lymphatic hypoplasia or aplasia in the periphery and even centrally. The left-sided lymphovenous valve (and the frequent second right-sided lymphovenous valve) connecting the thoracic duct (which is the central lymphatic collector) to the central vein is the only place (other than the much smaller right lymphatic duct valved entry) where the post nodal lymph fluid and blood normally come into contact. This may be the first 'lymphatic' valve to develop. The mechanotransducer PIEZO1 senses the laminar flow of lymphatic fluid and this detection is a major stimulus to valve formation[97]. Recessive mutations in *PIEZO1* are associated with human lymphoedema[98]. The precursor cells also require transcription factor SOX18, NRF2, Coup transcription factor 2 and PROX1. These lymphovenous valves continue to develop with FOXC2 as the major activator[99] for valve development, with planar cell polarity gene products and connexins in their maturation[100–102].

Planar cell polarity refers to the coordinated orientation of cells in epithelia in the direction perpendicular to the apical-basal orientation and is essential for cell orientation. In mice, two chemically induced, nonsense mutations in *Celsr1* were found to affect planar cell polarity (*spin cycle* and *crash* mouse mutants). These two mutants and a conditional deletion of *Celsr1*, using a *Prox1* promoter to specifically delete *Celsr1* in endothelial cells of developing lymphatics, allowed study of the role of CELSR1 in later development[103]; it was shown, with VANGL2, to have a crucial role in lymphatic valve formation[103]. Families with lymphoedema due to mutations in the planar polarity gene *CELSR1* have been described[29,104].

Gap junction molecules (connexins) are also important for lymphatic development and three have been found to be expressed in most lymphatic vessels: Cx37, Cx43 and Cx47 (REFS[105–107]). Knockouts of *Cx37* and *Cx43* disrupt lymphatic valve development and result in embryonic lymphoedema and chylothorax with markedly reduced postnatal survival[105]. Two *GJC2* mutations were initially reported in two families with dominantly inherited lymphoedema[108], followed by other families and one family with a mutation in *GJA1* (encoding Cx43)[33]. In addition, mutations in *EPHB4*, encoding a member of the ephrin family of RTK receptors, which interact with connexins, have been found in cases of fatal NIHF[109] (FIG. 3).

Expansion and proliferation of lymphatics. After the initiation of lymphangiogenesis, laminar fluid flow and interstitial pressure trigger lymphatic expansion and proliferation, at least in vitro. LECs exposed to laminar fluid flow stimulate a pore subunit of the s-activated calcium channel (ORAI1), which induces the upregulation of Kruppel-like factor 2 (KLF2) and KLF4 and induces the expression of VEGFA, VEGFC, fibroblast growth factor receptor 3 (FGFR3), and p57 (also known as cyclin-dependent kinase inhibitor 1C)[110]. As with most of the genes mentioned in initiating the development of lymphatics (excluding *VEGFC* and *VEGFR3*), mutations in these genes causing human lymphoedema have not yet been found. However, recessive mutations in *PIEZO1* are associated with human lymphoedema[98]. In addition, in a large multigeneration family with highly penetrant lymphoedema, digenic inheritance has been documented with both *FOXC2* and biallelic *PIEZO1* mutations[111].

Gap junction (connexin) proteins are not only involved in valve formation (Cx37 and Cx47) but are important for the function of lymphatics (Cx26) as they control the flow of fluid containing small and larger molecules between cells, which may be their role in valve formation. It is the leaked fluid from the blood vascular system that the lymphatics return to the blood circulation[112]. Gap junctions are also important in coordinating smooth muscle-mediated contractility, which propels lymphatic fluid centripetally[105,106,113].

Chromosomal loci associated with PLE. Several chromosomal disorders are associated with lymphoedema (TABLE 1). Turner syndrome (45,XO) frequently has infantile generalized lymphoedema (before the chromosomal cause was discovered, it was considered a separate syndrome, known as Bonnevie–Ullrich) and can re-occur in children and adults. Although an X-chromosomal p11.4 location shared with Yp11.2 has been identified, a specific gene has not been pinpointed[114,115]. Moreover, trisomy 21 (and the rarer trisomies 13 and 18) is frequently associated with increased nuchal folds detected in utero by ultrasonography (posterior lateral neck swellings thought to be related to enlargement of the cervical lymphatic sacs)[116]. Old case reports based on classical karyotyping associated mosaic trisomies or interstitial deletions and duplications to nuchal translucency or PLE[117–119]. The cause of these phenotypes could be a mutation in one of the now known genes) localized in these regions (TABLES 1,2). The frequently diagnosed Prader–Willi syndrome, which involves abnormal imprinting of a portion of chromosome 15 caused by

gene or chromosomal mutations, often presents with lymphoedema along with other syndromic features[55]. Additional loci include the locus of the Phelan–McDermid syndrome (22q13), the 22q11.2 deletion syndrome locus[120] and the locus for the Aagenaes syndrome (15q26.1)[121]. As the incidence of lymphoedema in various syndromes is unknown and case reports are scarce, epidemiological studies are needed.

Signalling pathways

Lymphangiogenesis. In addition to the proteins and signalling pathways implicated in human disease discussed above, there are many other signalling pathways involved in lymphatic development and function[122]. These pathways control cell growth and proliferation, apoptosis, cell migration and differentiation, and cell adhesion. As mentioned, VEGFC is essential for the initial development and maintenance of lymphatics, whereas VEGFD, which stimulates adult lymphangiogenesis by binding to VEGFR3, is not essential[123], at least in mice. The levels of VEGFR3 are strongly controlled by Notch signalling[124]. VEGFC stimulation results in receptor phosphorylation and downstream activation of multiple signalling pathways, which stimulate LEC proliferation and migration. Ephrin B2 signalling at its tyrosine kinase-activating receptor, ephrin type B receptor 4 (EPHB4), is also essential for lymphatic development (FIG. 3). This signalling pathway is unusual in that it involves 'reverse signalling', in which the ligand (ephrin) also functions as a receptor in the cell expressing it. This dual ligand–receptor function of the membrane protein mediates bi-directional signals between neighbouring cells; thus, intracellular signalling is induced in both cells (forward (in the neighbouring cell) and reverse (in the ligand-expressing cell)). The reverse signalling is essential for lymphatic remodelling and valve formation[125]. The ephrin signalling pathway as well as VEGFC and VEGFD signalling through neuropilins provide a connection between lymphatic network patterning and that of neurons. Vascular growth factors are secreted by neurons and neurotropins are secreted by developing vessels, enabling co-tracking of the development of both systems. Other signalling molecules, including semaphorins and Slits as well as their receptors plexin and roundabout homologue (Robo), also connect the two guidance pathways[126]. Their full role is beyond this Review but well covered in REF.[126].

Another signalling pathway important for lymphatic development is that of the ANGPT1 and ANGPT2 ligands and the tyrosine-protein kinase receptors TIE1 and TIE2. The two tyrosine kinase receptors are differentially activated by the two angiopoietins. ANGPT1 activates TIE2, whereas ANGPT2 activates TIE2 only on LECs and blocks the activation of TIE2 on angiogenic blood endothelial cells because an inhibiting vascular endothelial protein tyrosine phosphatase (VEPTP) is expressed in blood endothelial cells but not LECs[127]. Knockout mouse models demonstrate that ANGPT2 is required for haemangiogenesis and lymphangiogenesis, with the lymphatic defects being corrected by the expression of ANGPT1 instead of ANGPT2 (REF.[69]). Recently, loss-of-function or dominant-negative mutations were identified in *ANGPT2* in PLE[23].

Mutations in many genes of the RAF–MEK–ERK — MAPK cascade cause lymphoedema-associated syndromes. In mice, another pathway dependent on MAPK that is associated with lymphatic problems is the ternary complex factors pathway, which regulates immediate early genes through serum response elements. The knockout of the gene encoding one of these factors, *Net*, results in lymphovascular defects, including chylothorax[128], but PLE-causing mutations in the corresponding human gene are not known. Finally, hepatocyte growth factor (HGF) and its tyrosine kinase-activating receptor MET promote lymphatic vessel formation and function[129] (FIG. 3).

Overgrowth syndromes involving the lymphatics. A limited number of overgrowth syndromes involve the lymphatics. These syndromes can be quite disfiguring and, because germline mutations would be lethal, are due to somatic mutations. The famous case of the 'Elephant Man' involved an individual who was long thought to have neurofibromatosis type 1, whereas almost certainly he had Proteus syndrome, which is due to somatic, gain-of-function mutations in *AKT1*. The protein product of this gene, RAC-α serine/threonine-protein kinase (AKT1), is involved in a signalling pathway involving several genes implicated in lymphatic malformations. *PIK3CA* produces phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit-α isoform, which can be dephosphorylated by PTEN. This dephosphorylation decreases PI3K concentrations and prevents it from translocating AKT1 to the cell membrane, where AKT1 is phosphorylated and activated by upstream kinases. *PTEN* loss-of-function mutations cause PTEN hamartoma tumour syndrome, whereas somatic gain-of-function mutations in *PIK3CA* are found in CLOVES syndrome, characterized by congenital lymphatic overgrowth, vascular malformations, epidermal nevi and skeletal abnormalities, and in Klippel–Trenaunay–Weber syndrome of bone and angiolymphatic overgrowth as well as in isolated lymphatic malformations[55,76,130]. Other *PIK3CA*-related overgrowth syndromes that may be associated with lymphatic anomalies also exist[130].

In conclusion, our understanding of the pathophysiological mechanisms underlying PLE is incomplete. Nonetheless, molecular discoveries over the past two decades have identified multiple genes, proteins and signalling pathways involved in lymphatic growth and development, with these findings providing fundamental insights into PLE (FIG. 3). Further lymphatic imaging, particularly dynamic studies since the 1970s documenting the various peripheral and central lymphatic system disturbances in PLE, has served to connect the proposed molecular events with physiological evidence of lymphatic maldevelopment and dysfunction manifested in the clinical appearance and complications of PLE. Opposite pathophysiological pathways are reflected in the hypoplastic form (inadequate peripheral lymphatic growth) and hyperplastic refluxing form (defective lymphatic valve formation and central lymphatic malformation) of PLE depicted in FIG. 2.

Diagnosis, screening and prevention

Diagnosis

PLE is often diagnosed on the basis of a clinical examination. When swelling of the limbs and external genitals are found congenitally, in children or adolescents, with or without a syndrome, an anomalous lymphatic developmental disorder or syndrome should be considered (FIG. 4; TABLE 1). Signs and symptoms in different organs give clues to the diagnosis. Lymphoedema swelling can be mild to severe; worsen, fluctuate or improve with time; affect different parts of the body (limbs, arms, hands, head and neck, abdomen, etc.); be unilateral or bilateral; and present at different ages of onset even in adults. Lymphoedema leads to tissue changes such as an increase in fat or fibrosis over time. This observation explains why the pitting oedema clinical test (indentation from pressure applied with the thumb on a small area of the skin that persists after release of the pressure), which indicates the presence of interstitial oedema if positive, can be negative. The International Society of Lymphology has defined four clinical stages for lymphoedema[60] (BOX 1).

Multiple PLE phenotypes are possible, related to the organs affected by the failure of the lymphatic system such as lung effusions, intestinal lymphangiectasias (overt proliferation of lymphatic vessels) with chylous ascites and numerous embryonic oedemas under the term NIHF. It can be part of a complex syndrome, in which other signs concern the patient and family more than the lymphoedema. These conditions are rare and require a dedicated multidisciplinary workup. The European Reference Networks are established to provide cross-border health care for all EU citizens regarding rare and complex disease conditions.

Patients with PLE are reported all over the world. In tropical countries, where filariasis and podoconiosis (which is caused by chronic exposure to volcanic red clay soil) are the major causes of lymphoedema, PLE is probably often diagnosed as secondary lymphoedema. In developed countries, diagnosing PLE is becoming less ambiguous, allowing preliminary statistics of incidence and prevalence. Swelling of a limb in children is rare and requires specialized diagnosis. In one study, among 170 individuals aged <20 years referred under the diagnosis of lymphoedema, 25% had other diagnoses such as lymphovascular malformation, lipofibromatosis or lipoedema[131].

Testing functionality of the lymphatics

Isotopic lymphoscintigraphy has been used for >50 years and follows the migration of an isotopic tracer along the lymphatic vasculature after an interstitial injection[132]. Abnormal findings include delayed transit time of the radiolabelled colloid to the regional lymph nodes, dermal backflow (accumulation of tracer in cutaneous lymphatics), asymmetrical node uptake, formation of collateral lymphatic channels, and tracer uptake in deep lymph nodes in the elbow or knee region, which is

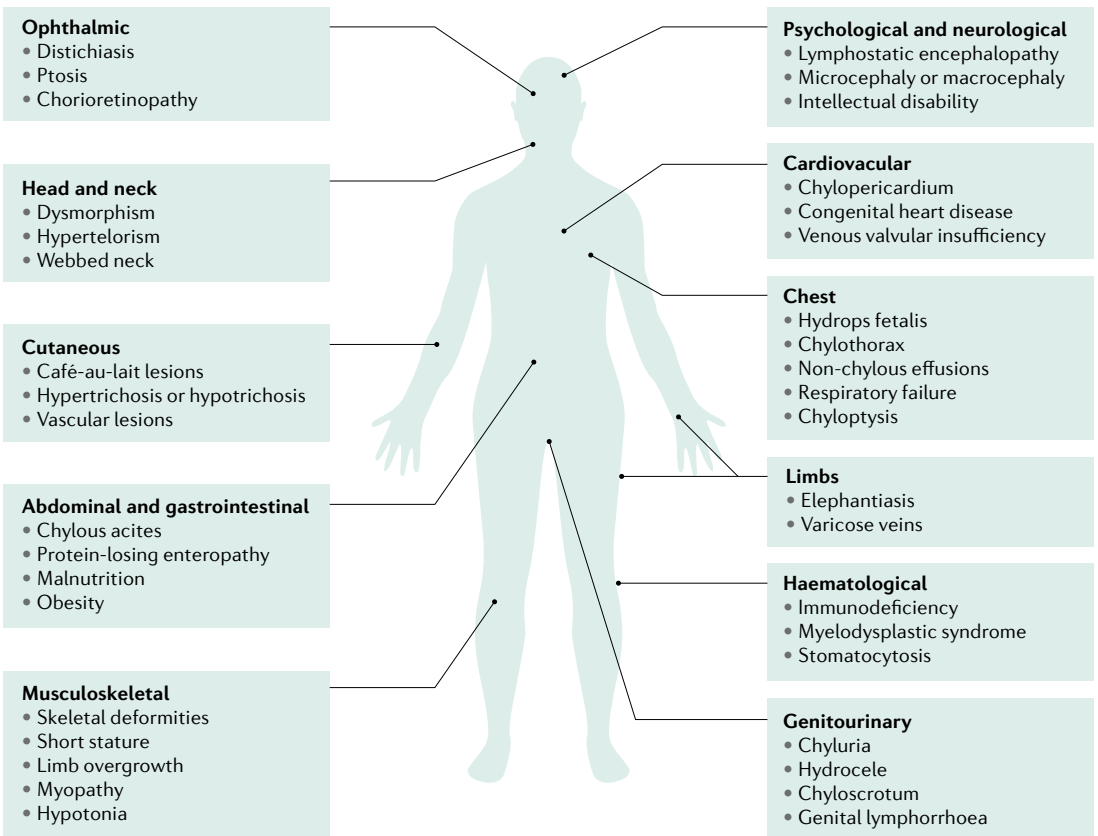

Fig. 4 | **Recurrent manifestations associated with rare syndromic PLEs.** The most frequently associated signs and symptoms of primary lymphoedema (PLE) are regrouped by organs and anatomical localization. See text for details and TABLES 1 and 2 as well as REF.[72].

pathological. The spatial resolution of scintigraphy is poor and has been improved using simultaneous anatomical localization by SPECT–CT. The isotopic lymphoscintigraphy patterns of gene-related PLE vary, from no intake in *VEGFR3*-related PLE to intake into large and numerous collateral lymphatics with dermal backflow in *FOXC2*-related PLE[33,65,109,133–135]. ICG fluorescence lymphography is used to evaluate the real-time transport of a fluorescent tracer in the lymphatic vessels in the upper dermal space up to a maximum depth of 3–5 mm (REF.[136]).

Imaging the lymphatics

Nowadays, non-contrast magnetic resonance lymphangiography (MRL; also known as lympho-MRI)[25] is a non-invasive technique that enables visualization of slow-moving non-bloody fluids such as those in large lymphatic vessels. It is based on heavily T2 weighted fast spin-echo sequences and maximum intensity projection reconstruction (FIG. 1). MRL has enabled the classification of the lymphatic system abnormalities in primary lymphoedema, considering lymph nodes and lymphatic vessel involvement[137]. Dynamic contrast-enhanced MRL enables static and dynamic visualization of the central lymphatic system by injecting gadolinium contrast agent in the groin lymph nodes in patients with PLE and/or CLA[138] or intrahepatic lymphatic anomalies[139]. This technique enables understanding of the lymphatic flow disorders before planning interventional procedures[140]; however, it is not widely available.

Pedal lymphangiography imaging using oil-based iodinated agents injected into the lymphatic vessels, which were dissected and cannulated, was developed for surgical purposes and used for the anatomical classification of PLE[141] but has been abandoned because of its viscosity and side effects. Lymphangiography based on the puncture of lymph nodes in the groin is now preferred to injection in the feet for the visualization of the central lymphatic conducting vessels in adults and children[142].

Box 1 | **The four clinical stages for lymphoedema as defined by the International Society of Lymphology**

A limb may exhibit more than one stage, which may reflect alterations in different lymphatic territories.

Stage 0 (or Ia)
Latent or sub-clinical condition in which swelling is not yet evident despite impaired lymph transport, subtle alterations in tissue fluid and/or composition, and changes in subjective symptoms. It may exist months or years before overt oedema occurs. This assessment requires imaging techniques.

Stage I
Early accumulation of fluid relatively high in protein content (for example, compared with venous oedema) that subsides with limb elevation. Pitting may occur. An increase in various types of proliferating cells may also be observed.

Stage II
Limb elevation alone rarely reduces the tissue swelling and pitting is manifest. Later in stage II, the limb may not pit, as excess subcutaneous fat and fibrosis develop.

Stage III
Comprises lymphostatic elephantiasis (enlargement of the limbs) in which pitting can be absent and trophic skin changes, such as acanthosis (overgrowth of the keratinocyte layer of the skin), alterations in skin character and thickness, further deposition of fat and fibrosis, and warty overgrowths, have developed.

Genetic analyses

Genetic testing is being used as part of the clinical workup in highly specialized centres managing patients with lymphatic anomalies, especially for familial cases. In genetic centres, most often, next-generation sequencing techniques are used to screen blood-derived DNA using gene panels. However, with a growing number of known genes related to PLE to be tested, whole-exome sequencing is becoming the best option. Chromosome analysis can be performed in detecting some syndromic PLEs. Tissue culture from affected tissue and next-generation sequencing can be helpful in finding somatic mutations but, so far, in most of the patients with PLE in whom a causative mutation has been identified, the mutations were germline (inherited or de novo). Yet, many patients remain undiagnosed and untested as they do not consult a specialized centre. Genetic testing increases our knowledge on phenotypic variability and genotype–phenotype correlations, enabling more specific genetic counselling as well as better stratification into subphenotypes and patient information.

Classification of lymphatic anomalies

A diagnostic algorithm based on detailed phenotyping, family history, age at onset, localization of the affected lymphatics, presence of visceral involvement, diagnosis of concomitant syndromes and genotyping has been established[15]. We propose here a revised classification of lymphatic anomalies (FIG. 5). This classification, although not complete as the field evolves rapidly, allows stratification of patients into main subcategories and considers the evolution of phenotypes in time (more signs become apparent with age). It also clearly links CLAs and lymphatic malformations to the diagnostic workup. Eventually, this approach can have therapeutic consequences and can prevent unnecessary (invasive) diagnostic measures[143]. Genetic data allow further refinement of this algorithm.

Screening

Screening for patients who are at risk of developing PLE is difficult in terms of selection. Three groups are at risk: relatives of a patient known to have PLE, patients with one of the syndromes associated with PLE but without clinical signs or symptoms of PLE, and patients who develop erysipelas (an infection of the superficial layers of the skin) without any preceding signs of chronic oedema, chronic venous insufficiency, lymphoedema, diabetes mellitus, overweight or previous bouts of erysipelas[144]. There are no guidelines on pre-symptomatic screening and we lack estimations for the risk of developing PLE.

When a patient is diagnosed with a gene defect causing lymphoedema, the possibility for family screening can be offered. Full information for the patient and consideration of the advantages and disadvantages are needed for shared decision-making. Owing to variable penetrance, the lymphoedema can be mild or even absent, which influences the relevance of screening for relatives. Despite all developments in the field of genetics and sophisticated techniques to visualize the anatomy and function of lymphatics, the role of clinicians within an

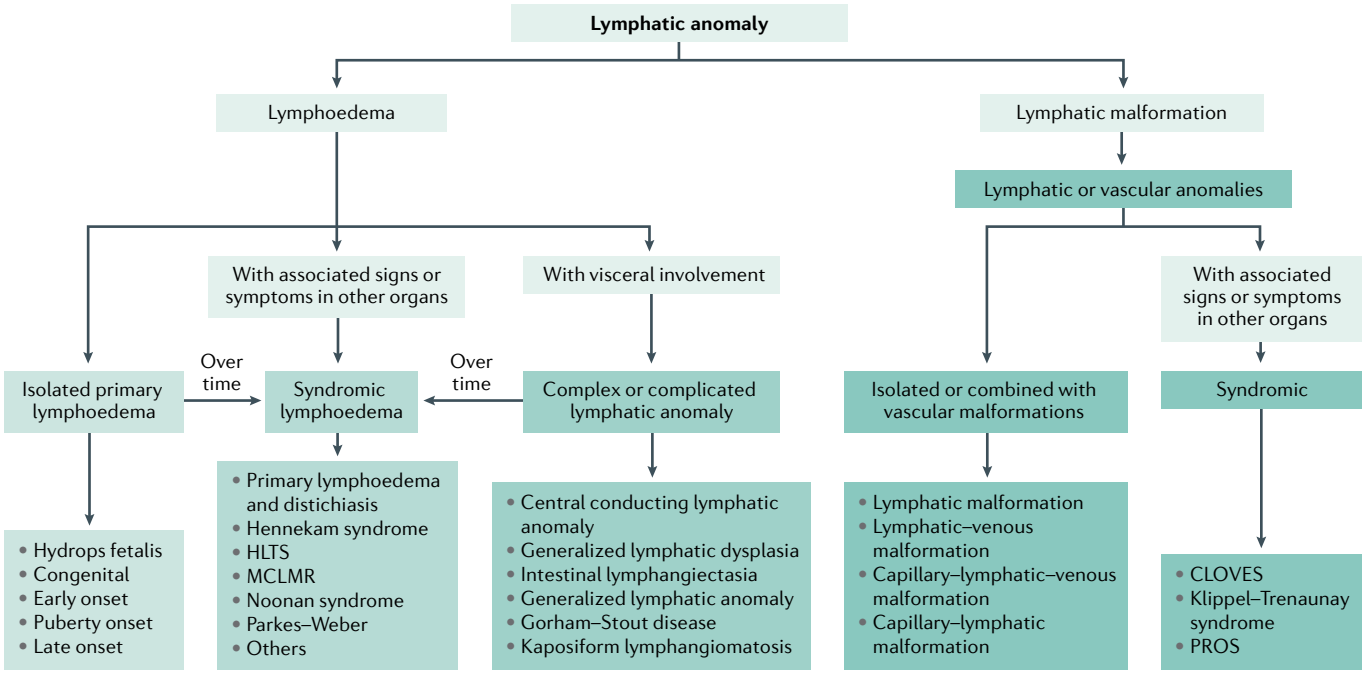

Fig. 5 | **Proposed classification of lymphatic anomaly phenotypes.** This practical algorithm allows the stratification of patients into main subcategories and considers the evolution of phenotypes in time (more signs become apparent with age). It also clearly links complicated lymphatic anomalies and lymphatic malformations to the diagnostic workup. Isolated primary lymphoedema (PLE) can have different time points of symptom onset; in isolated PLE, only peripheral lymphoedema (with or without varicose veins) is present. If signs or symptoms occur in other organs, diagnosis is more likely to be a lymphoedema-related syndrome (see TABLE 1 for genes). Complex or complicated lymphatic anomalies include phenotypes in which lymph and/or chyle accumulate centrally in the trunk, including, for example, chylous ascites, pleural effusions and intestinal lymphangiectasias (see BOX 2 for genes). Lymphatic lesions that are more localized are defined as lymphatic malformations and, in these conditions, lymphoedema is rarely present. As phenotypes evolve postnatally, a diagnosis may move from isolated PLE or complex or complicated lymphatic anomalies towards syndromic lymphoedema. Diagnostic terms are those used by ISSVA (International Society for the Study of Vascular Anomalies). CLOVES, congenital lipomatous overgrowth with vascular malformation, epidermal nevi and scoliosis syndrome; HLTS, hypotrichosis–lymphoedema–telangiectasia syndrome; MCLMR, microcephaly with or without chorioretinopathy, lymphoedema or mental retardation; PROS, *PIK3CA*-related overgrowth syndrome. Adapted from REF.[15], CC BY 4.0.

interdisciplinary expert team is crucial for meticulous phenotyping, selection of diagnostic tools and use of genetic techniques[145].

Patients experiencing one episode of erysipelas of the leg, which presented without warning signs and without signs of previous lymphoedema, frequently (79%) show lymphatic impairment of both legs by scintigraphy[30,146]. In daily practice, bilateral scintigraphy can be useful to confirm lymphatic impairment.

Prevention

Preventive medicine is often used in chronic conditions in which an overall cure is not possible. Three categories are recognized: primary prevention, focusing on preventing the disease in the general population; secondary prevention, intended for those with risk factors but clinical signs or symptoms not yet observed; and tertiary prevention, which is part of the treatment of active disease. The interventions used for these three types of prevention differ.

For relatives of patients with PLE who carry the gene defect but are asymptomatic, secondary prevention can be relevant to prevent lymphoedema. Genetic testing can give a decisive answer about the risk in such cases. When symptoms of PLE are present, tertiary prevention is important to support treatment regimens and to try to minimize the negative impact of the disease, improve function and prevent complications. No study has been performed for PLE only.

The interventional parts of secondary and tertiary prevention have many elements in common, including staying active and maintaining a healthy lifestyle with enough physical exercise[147–149], preventing obesity at all ages[150,151] and preventing erysipelas[3]. In secondary prevention, clinimetrics (indexes, rating scales and other expressions used to describe or measure symptoms, physical signs and other clinical phenomena) for lifestyle (for example, pedometer, weight control) are performed by the patients themselves and there is no concomitant treatment related to lymphatic vascular diseases. In tertiary prevention, intervention is part of the treatment protocol for PLE and is monitored with clinimetrics in the International Classification of Functioning, Disability and Health (ICF) domains[131,152].

Management

Non-invasive medical and conservative management

Long-term management in all patients aims to minimize the negative impact of the disease, improve function, and prevent short-term and long-term complications. Considering that PLE is a rare and chronic condition, attention is also paid to holistic management

and long-term patient self-management. From a therapeutic perspective, much research has focused on breast cancer-related secondary lymphoedema. These recommendations include wearing garments, skincare, and preventing personal factors that may mistakenly consider lymphoedema as weight gain or obesity[153–155] or due to the lack of physical activity[149,150]. Although not studied for PLE, these factors can be extrapolated to be relevant for PLE. To further increase our knowledge, management of PLE should also include the identification of associated genetic mutations. Selected patients might benefit from gene-targeted drug therapies, especially within the group of CLAs (BOX 2). In tropical countries with low resources, the WHO recommends daily hygiene practices, such as washing and drying the skin to avoid infection, as the central component of long-term self-care for the management of filariasis-related lymphoedema, with limited benefit on swelling. Compression, a key component to reduce and prevent worsening of swelling, is commonly not available because of limited health-care resources. Similar considerations are expected to apply to PLE.

Control of swelling. PLE swelling occurs in any part of the body, although it affects the lower limbs more frequently[131,156]. The prevention of worsening of swelling and tissue changes is mainly achieved through the application of compression, prevention of skin infections and controlling weight (FIG. 6a–d; Supplementary Fig. 1a–g). Complex decongestive therapy aims at reducing swelling within a few weeks using multilayer compression bandaging, manual lymphatic drainage, skincare, decongestive exercises under compression and rehabilitation. Maintenance therapy aims to maintain the reduction of swelling long term after intensive complex decongestive therapy or can be the only therapy when swelling is mild, based on a randomized study in patients with cancer[157] involving long-term wearing of compression hosiery, either regular or tailor-made, skincare, and exercise. There are few clinical practice guidelines[158] (of which only two are international, by the International Lymphedema Framework and the American Venous Forum[159,160]) or international consensus statements[55,60] and none specifically focuses on the management of PLE.

Conservative therapy can now be tailored according to natural history of PLE when the genotype is known. As an example, swelling of the *VEGFR3*-associated Nonne–Milroy disease remains localized under the knees, mainly involving the forefoot, toes, ankles and the leg under the knee. Care focuses on distal bandages, skin moisturization, and prevention of ingrowing nails and related infections. The end of uncertainty about the future for these patients positively affects their quality of life.

In other cases, venous insufficiency due to incompetent venous valves, such as in lymphoedema–distichiasis[161] or oedema secondary to hypoalbuminaemia[42], may hamper swelling control. Specific approaches are used in patients with PLE of the genitals as effective compression is difficult and debulking surgery usually takes place early[162,163].

Data related to compression use in children with lymphoedema are rare[164–166]. There is a soft agreement on avoiding applying bandages or hosiery systematically on babies' limbs as long as function and mobility are not impaired as stated on the VASCERN guideline adapted from the French national guideline[42].

Treatment of lymphatic-related organ failure and associated syndromic comorbidities. Intestinal lymphangiectasia results in an exudative enteropathy with hypoalbuminaemia and subsequent worsening of swelling and γ-globulin deficiency. The cornerstone of management relies on a specific low-fat diet excluding the long-chain triglycerides that are absorbed by intestinal lymphatics and associated with drugs that reduce chyle flux and loss of albumin[167]. Infusion of γ-globulin and albumin is required in severe cases. Pulmonary lymphangiectasia or reflux result in restrictive respiratory failure. Prevention of infections and oxygen supply are the only effective treatments. Syndromic comorbidities, such as learning difficulties in Phelan–McDermid syndrome[168] or sensory-neural deficiencies in Emberger syndrome[169], can make the application of compression therapy difficult. Growth hormone use in children with Noonan syndrome has no effect on lymphoedema itself[170]. Diseases such as Emberger syndrome are also associated with a specific risk of leukaemia or cancers; patients should be monitored through cancer surveillance programmes[171,172] and benefit from haematopoietic stem cell transplants early in life[172–176]. In the case of CLAs (BOX 2), lymphatic organ failure associated with pulmonary and intestinal lymphangiectasia, pleural or pericardial leakage or ascites, and bone destruction requires specific interventions.

Infections. Complications such as cellulitis starting early in life[3], sometimes even before lymphoedema manifests[146], require treatment with antibiotics (usually penicillin). Long-term prophylaxis of recurrences or early self-initiated antibiotic treatment are proposed, along with careful skincare and treatment of fungal infections[144]. Warts are described in children[177], are frequent in specific PLEs such as GATA2 deficiencies[178–180], and may require specific treatment[181,182].

Repurposing cancer drugs for CLAs. In a few patients with a CLA, a somatic gain-of-function mutation that activates an intracellular signalling pathway also

Box 2 | **CLAs or lymphangiectasias**

Complicated lymphatic anomalies (CLAs) are characterized by localized lymphatic malformations affecting bones and other tissues with chylothorax, chylopericardium and/or chylous ascites. Primary lymphoedema is present in some cases. Examples include the following.

- Generalized lymphatic anomaly, caused by a mosaic gain-of-function mutation in *PIK3CA*[185]
- Gorham–Stout disease, caused by a mosaic gain-of-function mutation in *KRAS*, possibly by a germline loss-of-function mutation in *PTEN*[187,243,287]
- Kaposiform lymphangiomatosis, caused by a mosaic gain-of-function mutation in *NRAS*[183,184]
- Generalized lymphatic dysplasia, caused by recessive mutations in *ADAMTS3*, *CCBE1*, *FAT4*, *FBXL7*, *PIEZO1* (REFS[93,235,236,238,246,247,275])

CLAs that can be associated with primary lymphoedema include central conducting lymphatic anomalies, for example, RASopathies and Hennekam syndromes[188,189,237,288].

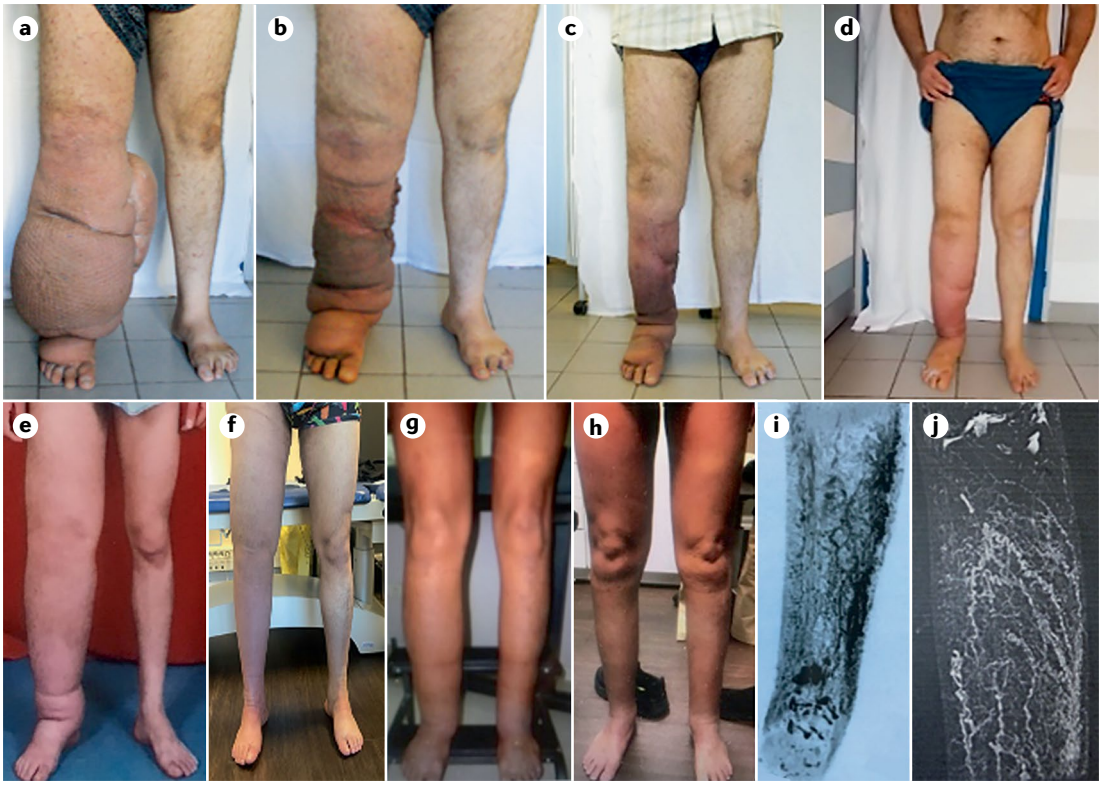

Fig. 6 | **Non-surgical and surgical treatments of PLE.** Patient with primary lymphoedema (PLE) since adolescence. Situation 9 years after partial debulking surgery without follow-up compression (part **a**); after 2 months of intensive decongestive treatment with multilayer bandages (part **b**); and after 10 months (part **c**) and 27 months (part **d**) of self-management. Lateral and back views in Supplementary Fig. 1. Patient with PLE since birth (part **e**) was operated by lymph node transfer in the inguinal area and, 1 year later, in the knee region; results after 4 years (part **f**). Liposuction was performed on the thigh. Compression garments were still needed. Patient with PLE since birth, associated with chylothorax in childhood (part **g**); result 1 year after lymphovenous anastomosis (part **h**). Lympho-MRI before (part **i**) and after lymphovenous anastomosis (part **j**).

implicated in cancers has been identified[183–187]. Central conducting lymphatic anomalies (CCLAs) are also not infrequently observed in various RASopathies, such as Noonan syndrome, owing to activation of the RAS–MAPK signalling pathway. Thus, repurposing of small-molecule inhibitors developed for oncology to target these pathways has emerged as a novel possibility to test for the treatment of selected patients with CLAs[188,189].

One of two patients with CCLA and a gain-of-function *ARAF* mutation was treated with the MEK inhibitor trametinib off-label. Improvement of lymphoedema and pulmonary function was observed[190]. Similarly, a patient with Kaposiform lymphangiomatosis with a somatic *CBL* variant seems to have benefited from treatment with a MEK inhibitor[186]. Increased survival and reduced lymphatic backflow was observed in a mouse model of Gorham–Stout disease treated with trametinib. The model was generated on the basis of an activating *KRAS* mutation identified in Gorham–Stout disease[187].

Aside from these cancer drugs, the development of drugs targeting inflammation, such as bestatin, an inhibitor of leukotriene A4 hydrolase (LTA4H), was investigated in patients with either secondary or primary non-congenital lymphoedema suggesting some positive effects[191]. However, the trial was stopped because bestatin demonstrated no improvement over placebo in the primary endpoint of skin thickness and secondary endpoints of limb volume and bioimpedance.

Interventional procedures

Surgery. Two kinds of surgical procedures are used in patients with PLE: those aiming to remove excess tissue and those aiming to restore lymph flow. Radical excision of skin and subcutaneous tissue to the level of the deep fascia followed by skin grafting is no longer used on legs owing to poor aesthetic and functional outcomes. Nowadays, the main indication for excision surgery is to treat primary scrotal lymphoedema, for which compression often fails with severe psychological impact[27].

Liposuction removes excess fat tissue, which is part of the swelling of primary lymphoedema[192,193]. It has been proposed as an alternative to the removal of tissue in paediatric patients[27]. It is a second-line option in patients with psychosocial distress because of the appearance of the affected area, recurrent infections and substantial impairment of function[194]. The level of evidence for positive outcomes is increasing[193,195,196]. Lifelong high levels of compression are required around the clock to maintain

the benefit. Liposuction is not indicated in patients seeking an alternative to compression or not complying with lifelong compression. Bariatric surgery improves limb volumes in patients with obesity with PLE[197].

Lymphatic reconstructions aim to restore lymphatic flow and are proposed earlier during lymphoedema. Microsurgical procedures create lympho-lymphatic and lymphovenous anastomosis. These techniques have been proposed for >30 years, mostly in carefully selected groups of patients with local segmental obstruction of proximal lymphatics (for example, following cancer treatment) when peripheral lymph vessels were patent and with preserved contractility[198]. Supramicrosurgical reconstruction enables the creation of fine connections (0.3–0.8 mm in diameter) between distal lymphatics and subdermal venules (lymphaticovenular anastomosis). These techniques present good results if the lymphatic vessels are of good quality (autonomous contraction and valves are still present) (FIG. 6g–j). Guidance with lymphoscintigraphy patterns and ICG fluorescence lymphangiography are being investigated to screen proper candidates and choose the dynamic vessels[199–201]. Only a few observational non-randomized studies include patients with PLE of lower limbs or genitals despite optimized compression decongestive management[200,202,203]. These approaches might not be useful in *FOXC2*-associated PLE or PLE associated with mutations in connexin genes as the venous system presents reflux or there is absence of contractility of functional capillaries[100].

Free lymph node transfer (LNT) is based upon the transfer of a group of nodes and their own vessels from an unaffected extremity to a new location. It is a fascio(cutaneous) flap with arteries and veins that is transferred and revascularized by microsurgical anastomosis, like finger transplantation. In hypoplasia cases, the addition of nodes in a free fatty flap (autologous lymph node transplantation from patients themselves, avoiding graft rejection) enables neogenesis of lymphatic vessels owing to the presence of growth factors (VEGFs and cytokines). LNT and autologous lymph node transplantation are reconstructive techniques that have been developed mainly as prevention or treatment of secondary lymphoedema after breast cancer treatment. The available case reports testify complete normalization in 20% of patients[204] (FIG. 6e–f; Supplementary Fig. 1h–i). LNT has been associated with lymphoedema occurring on asymptomatic limbs in patients with PLE[205]. The combination of LNT with lymphovenous anastomosis is proposed in the management of complicated PLE in adults and children[206].

Embolization of the lymphatics. The improvement of static and dynamic lymphangiography techniques enables the diagnosis of lymphatic flow anomalies and the guiding of lymphatic interventional procedures. Embolization of reflux from the thoracic duct into the pulmonary parenchyma and of leakages into the mesenteric lymphatics was first developed as a palliative option in selected patients with single ventricle heart malformation in whom heart transplantation could not be performed following failure of the surgical cavo-pulmonary circulation, known as the Fontan circulation[207,208]. The resulting elevated central venous pressure and high lymphatic flow lead to protein-losing enteropathy and plastic bronchitis (characterized by expectoration of branching bronchial casts) in some patients with additional lymphatic anomalies[209]. Interventional techniques, for example, catheterization of the thoracic duct and embolization, are increasingly used in the management of central conducting lymphatic anomalies associated with lymph and chyle reflux, such as chyluria[210], genital leakage[211], protein-losing enteropathy and ascites[212], and pulmonary lymphatic anomalies[46].

Quality of life

PLE is a chronic condition and its diagnosis is only one aspect of patient assessment. The large psychological burden of PLE and chronic oedemas has been evidenced using generic or specific health-related quality-of-life tools[12,213] and is worse in patients in hospital than in those treated at the community level[214], underscoring the necessity to include these aspects in the diagnosis and health profile assessment[215,216]. In 2001, the WHO introduced the ICF[217]. This bio-psycho-social ICF model includes five domains of functioning of a patient: biomedical aspects, activities of daily living, participation in society, personal factors and environmental factors; the latter two influence the first three functional aspects and need to be considered. Validated clinimetrics[218] can be established by a consensus group or during the creation of guidelines[152] to design a treatment protocol and the measurement of its effects in all domains.

Most studies utilizing ICF health profiles[219] and dedicated ICF quality-of-life questionnaires (such as lymph ICF-lower limb[220] and the Lymphedema Quality of Life Inventory (LYQLI)[221]) are based on secondary lymphoedema. A comparison of the validity of five different outcome measurement tools was performed in patients with lower-limb lymphoedema and suggested specific use according to the aim of the outcome measurement[222].

Thus, it is not clear whether extrapolation of the validity of quality-of-life tools used for secondary lymphoedema to PLE is acceptable. Because PLE is a rare disease, it is associated with delayed diagnosis, feelings of insecurity and mistrust of professionals, aspects that do not exist in secondary lymphoedema[223]. Growing with a different body shape also has different effects from secondary lymphoedema. Moreover, specific questionnaires are being developed for children and young adults (NCT01922635).

Long-term management of PLE issues

Decongestive lymphoedema therapy is the first line of management of PLE to improve quality of life. However, compression maintenance therapy of lymphoedema is a lifelong process and remains the main treatment even after interventional procedures. It requires that the patient wears compression garments daily, exercises, performs skincare, and sometimes repetitively conduct self-massage and bandaging. The burden of long-term management is obvious and requires a commitment to self-management and a self-efficacy belief as shown in children and parents[224]. Compression is not mandatory

Box 3 | **Patient perspective (part 1)**

E. is a 24-year-old young woman born with a generalized lymphatic anomaly. These are her words about what it means to live with primary lymphoedema, recorded on December 2020 as a webinar.

The journey for a diagnosis

I was 8 weeks old, my mum noticed that my left arm was bigger than my right arm, and they had no idea what was going on. There started a very long journey to diagnose it. We went to the general practitioner, who then referred diagnosis to a specialist paediatrician in the area. I was diagnosed with hemihypertrophy syndrome. At that time, 24 years ago, there was very little understanding of what was going on and my parents would feel a lot of confusion. It was only a few years later that they started to notice that it was my left arm and leg, but also that my lungs were involved. This led to being able to see a specialist team, who diagnosed pulmonary lymphangiectasia and determined that I was having a sort of underlying generalized lymphatic anomaly.

I could talk for ages about how long this journey took. The essence of what I am saying is that growing with this, having lymphoedema, was not really understood at all at that time. My parents talk about the challenges and difficulties they had to be able to access the care I needed and just to be able to understand what was going on. This uncertainty went on for many years, until we were able to get the right diagnosis many years after. Being able to access specialized centres with doctors and professionals who know what is going on makes a huge difference. That is so life-changing.

Growing up with lymphoedema

Ultimately, it is about understanding the needs of children and young people. They are quite different. Following the medical recommendations, you feel it is quite a big commitment. One of the hardest things for me was managing all of these different appointments, having to travel to a lot of different places and seeing many different doctors. At the same time, working at school and getting my degree was so important to me. I struggled a lot with not being at school because of lymphoedema.

Being now 24 years old, my advice would be to rely on a support network and get plans in place with school, colleges and universities. Being open to the reality of the challenges, and this is not easy to do. It is not easy to stand in front of people and talk about your challenges in life. In the end, it is about understanding that people can help you if they understand how they can help you. For example, [finding] shoes, clothes, garments, that is a daily struggle, and I still find that so challenging. One day hopefully I will get a pair of red high heel shoes; that is one of my aspirations in life, still a way to go.

in toddlers and even children might not cope with aggressive decongestive therapy or wear prescription garments. Professionals need to adapt compression to the patient's life and not vice versa and take the time needed for patients to accept their condition and tailor the therapy. Education can be delivered in camps or during social and networking activities; it helps young people not to feel alone with their rare condition and is also useful for professionals to share their experience[225,226] (BOXES 3,4).

Outlook

Lymphoedema is a global problem (BOX 5). The management of PLE is still in its infancy, although the disease has been recognized for years. One of the reasons is the wide variety of presentations, from isolated forms to combinations with other features in syndromes. A second major reason is the lack of understanding of the pathogenetic events leading to the disease and the underlying genetic causes.

Genetic variability

In large-scale genetic screens, numerous variants of unknown significance are detected. If these were to be considered as disease-causing mutations (assuming that they would be validated as such by functional tests), the proportion of individuals in whom a causative mutation is identified could be as high as 70% of patients according to our estimates (P.B. and M.V., unpublished work). Yet, most likely, the majority of variants of unknown significance do not have an effect on protein function and represent rare polymorphisms. Thus, novel genes associated with PLE need to be looked for as many pieces are still missing to reconstitute the full puzzle and finding additional pieces would lay down the basis for understanding the multiple reasons for the development of PLE.

Additional challenges in identifying the genetic basis of PLE are based on the fact that the available techniques to identify causative intronic and intergenic variants are less developed than those for exonic variants. As the effect of disease-causing variants should present at protein level and therefore also at the mRNA level, RNA-based studies might be helpful. This approach could provide insights on deep intronic mutations, exonic splice-altering mutations that are not identified as pathogenetic as they are synonymous changes, promoter mutations, methylation disturbances, and other changes that would regulate gene expression level. After an exhausting search for intragenic mutations, we could look for alterations in the rest of the genome: mutations in non-coding parts of the DNA, microRNAs, long non-coding RNAs or DNA alterations that result from viral infection for viruses that integrate their genetic material into the genome. Occasionally, patients with unilateral PLE could carry a somatic mutation only detectable in the affected tissue. Moreover, PLE could also develop as a result of gene-to-gene interactions and therefore validated algorithms to study oligogenic diseases are needed.

Many of the mutations in genes causing Mendelian forms of PLE have incomplete penetrance. Sex-specific penetrance is also starting to emerge and could help provide a more accurate prognosis when taken into consideration. Hormones seem to have an important role in PLE development yet their influence is poorly understood and needs focused studies.

Another intriguing question is the overlap of PLE-causing genes with secondary lymphoedema as the same genes may be involved. Two studies have suggested such a link[95,227]. An association was found with *LCP2* (encoding lymphocyte cytosolic protein 2), *NRP2* (encoding neuropilin 2), *SYK* (encoding spleen tyrosine kinase, also known as tyrosine-protein kinase SYK), *VCAM1* (encoding vascular cell adhesion protein 1), *FOXC2* and *VEGFC*. However, other studies are needed to confirm the significance of these associations.

When developing therapeutic approaches, it is important to visualize the interplay between the different genes that have already been identified for PLE. In 2014, 20 of them were grouped in a hypothetical LEC and many of the gene products were geared around a central VEGFC–VEGFR3 signalling pathway[74]. This model has now become more complex, with the inclusion of other ligand–receptor complexes (ANGPT2–TIE1/TIE2 and HGF–MET) and the effect of RAS–MAPK and PI3K–AKT signalling, which point to more severe syndromic forms (FIG. 3). Moreover, along the lymphatic vasculature, the impact of the gene mutations and mutated proteins

Box 4 | **Patient perspective (part 2)**

Fears

Fear and anxiety around cellulitis are very real. They can come on very quickly, they can be severe and you can feel very unwell. It is a constant in the back of your mind. You have to be aware of it, that it is dangerous. But you still have to live and enjoy your life. It is a balance. Now, I learned my warning signs, and that is important and takes time. It is also about getting help from the network you have built around you, including professionals and clinicians.

Covering the information with the general practitioner and self-management

As a patient, you can have so much control on your lymphoedema. I was the one sharing the knowledge with the general practitioner, using leaflets I was given by the support group. But with that comes a lot of responsibilities. You can in the end feel overwhelmed.

Psychological aspects

I would not cope with people seeing my leg for years. It took a lot of time to wear a dress when I wanted to wear a dress. We have to be in a place of self-acceptance. Lymphoedema is for life, and it takes time to understand and live with it.

I was not good at taking my medication and putting my compression on. Well, to be honest, we all get good and bad days. So it is also important that young people know that it is OK not to always feel OK with lymphoedema.

Sharing with peers

When you see other patients as a young person, there is no better feeling. You do feel alone with a rare disease, and it can be hard. It has been hard to establish my identity as 'E.' and not only 'E. with a bigger leg'.

may differ. Some mutations affect LEC proliferation or survival more globally, whereas others affect the development or stability of lymphatic valves or lymphovenous valves (such as *EPHB4* mutations). Thus, we will need in vivo models to gather more detailed data for the various pathways involved and unravel eventual ways to develop novel therapies and preventive measures.

Diagnosis and treatment

The diversity of genes associated with PLE, both known (10 of them were identified during the past 5 years) and still to be unravelled, combined with technological advancements, can lead to the rapid evolution of diagnostic genetic testing of PLE. Gene panels have given the most reliable results but they rapidly become outdated. The recommended diagnostic approach is moving towards whole-exome sequencing, for which the analysis can be re-processed for individuals in whom a causative mutation has not been identified when new genes are reported. With evolving copy number detection algorithms, whole-exome sequencing data can also identify partial and whole-gene deletions, although there are still limitations. Overall, increased germline genetic testing of patients will enable more precise (molecular) diagnostics and genetic counselling, which in turn allows at-risk family members to be informed and establishment of preventive measures. Importantly, such data help to better stratify patients for clinical research studies.

Detailed clinical analysis of all patients, including re-evaluation after established genetic diagnosis, will enable calculation of the risks for additional signs and symptoms in each PLE subtype. For example, in hypotrichosis–lymphoedema–telangiectasia syndrome (characterized by sparse hair, lymphoedema and small dilated cutaneous vessels), additional features are reported in almost every new case for this pleiotropic disease[228]. Such stratified epidemiological data will be paramount for the clinical diagnostics, genetic counselling, management and follow-up of patients. Such data will also help in the development of diagnostic algorithms[15] (FIG. 5). Stratification will also enable the more precise study of response to conventional and surgical treatments and, eventually, to predict outcomes, which could have an important impact on patient care.

Imaging technologies are also developing rapidly, promising a more detailed evaluation of PLE. MRL is now increasingly used, also in combination with peripheral ICG fluorescence lymphangiography. Combining these two techniques can help to reclassify the cause of PLE to peripheral, central or combined deficiency. Whole-body lymphangioscintigraphy associated with 3D localization by SPECT–CT can provide functional imaging of peripheral and central lymphatic flow[60]. Such multimodal approaches can be particularly useful in cases of PLE associated with CCLA and can guide the planning of interventional management. The Society of Interventional Radiology (SIR) and the *Journal of Vascular Interventional Radiology* selected lymphatic imaging as their focus topic of the year for 2020 (REF.[46]).

Photoacoustic imaging is another emerging imaging technique that provides unique scalability of optical resolution and acoustic depth of penetration. When associated with the use of light-absorbing biomolecules, such as oxy-haemoglobin and deoxyhaemoglobin and lipids, specific vascular structures can be targeted. Moreover, indocyanine green, which is taken up by lymphatic vessels, allows the use of photoacoustic imaging for lymphangiography, which has a higher spatial resolution than other imaging modalities[229–231].

The outlook for future personalized management of PLE is multifaceted. Diagnostics and interventional procedures should benefit from the further development of sensitive imaging methods for lymphatic dysfunctions. Combined with large-scale genetic analyses and other omics-type approaches in research, it should gather data to decipher the underpinnings of PLE development. Understanding PLE pathophysiology is the foundation

Box 5 | **Lymphoedema — the global picture**

Lymphoedema is globally a Neglected Public Health issue[6]. In upper-middle-income and high-income countries, where cancer is one of the top 10 leading causes of death, lymphoedema is recognized as a sequala of cancer, associated with a solid reputation of being an untreatable adverse effect of the treatment of cancer[11]. Its incidence is increasing with ageing, immobility and obesity, which are leading risk factors for lymphoedema and related cellulitis in these countries[289,290]. The WHO recognizes lymphatic filariasis as one of the five preventable neglected tropical diseases threatening 859 million people in 50, mostly low-income, countries. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) is aiming at providing anti-filarial medicines and a minimum package of care in all areas with known patients and progression of lymphoedema[291]. This package focuses on hygiene and misses the point of compression. PLE is hardly recognized in these countries[292] but has been recently recognized in Europe as a rare disease (prevalence <1 per 2,000 individuals[36]) and benefits from programmes dedicated to Rare Disease Networks such as VASCERN. Yet, unequal access to diagnosis and treatment is the reality all patients face owing to the underdiagnosis of lymphoedema, lack of efficient management and the inadequacy of the reimbursement policies for medical devices even in Europe[293]. Dedicated research programmes are required as stated in the USA by the Lymphatic Education & Research Network.

for the development of innovative PLE-specific therapeutics such as modulators of lymphangiogenesis and/or gene therapy approaches, stem cell-based treatment, and the development of artificial lymphatics by tissue engineering. In addition to bestatin, VEGFC has been used in a clinical trial. It was combined with LNT to induce lymph vessel growth and connectivity to the transferred lymph nodes in patients with secondary lymphoedema after breast cancer[232]. However, the company (Herantis Pharma) recently announced that the results were inconclusive. The eventual benefit of this technique, aiming to induce lymphangiogenesis locally, remains unknown in PLE. Targeted therapies have already emerged for other vascular anomalies caused by somatic mutations that increase signalling via the PI3K–AKT–mTOR pathway or the RAS–RAF–MAPK pathway, including CLAs[188]. Thus, the future is full of hope also for patients with PLE.

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